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ORIGINAL RESEARCH

The relation of serum prolactin levels and *Toxoplasma* infection in humans

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Background: *Toxoplasma gondii* is an intracellular protozoan parasite distributed worldwide. Although the infection is benign in immunocompetent individuals, it is life threatening and complicated in immunocompromised patients and fetuses of pregnant women who received their first exposure to *T. gondii* during the pregnancy. Prolactin (PRL) is a hormone that is secreted by the pituitary gland, and it is confirmed that it plays a role in the immune system. The present study was carried out to assess the possible relation between serum PRL levels and *Toxoplasma* infection frequency in human.

Methods: In this cross-sectional study, 343 serum samples (240 from women and 103 from men) were collected from individuals who were referred for PRL checking in laboratories of Karaj, Iran. Blood samples were collected, and sera were separated and analyzed for the detection of anti-*Toxoplasma* IgG antibody by ELISA method. The levels of PRL were measured by Roche Elecsys 2010 analyzer, electrochemiluminescence technology.

Results: Of 343 sera, 110 samples (32%) consisting of samples from 42 men and 68 women had anti-*T. gondii* IgG antibody. The prevalence of *T. gondii* infection in women with high PRL levels was lower than that in the comparison group with normal levels of PRL and the relationship between these two parameters was statistically significant (P=0.016). In women with hyperprolactinemia, by increasing of PRL levels, the prevalence of *T. gondii* infection was reduced.

Conclusion: The results of the current study confirmed the previous studies based on immunoregulatory role of PRL and indicated that high levels of PRL could be related to *Toxoplasma* seronegativity in women.

Keywords: *Toxoplasma gondii*, IgG antibody, prolactin, ECL technology, hypoprolactinemia, cytokines, hyperprolactinemia, dopamine

Background

Toxoplasma gondii, the protozoan parasite distributed worldwide, is common among humans and a broad range of warm-blooded animals.[1] The main routes of human infection are by the consumption of raw or undercooked meat containing tissue cysts and ingestion of oocysts via other food products, water, or vegetables.[2] Congenital infection can occur by vertical transmission of rapidly dividing *T. gondii* tachyzoites during pregnancy.[3] Prenatal infection leads to an increased risk of spontaneous abortion, chorioretinitis, or serious neurodevelopmental disorders such as hydrocephaly and microcephaly.[3] Although *T. gondii* infection is benign in immunocompetent individuals, it is life threatening in congenital form and in immunocompromised patients due to reactivation of the infection.[4] Therefore, accurate diagnosis of acute maternal toxoplasmosis in immunocompromised patients and pregnant women is critical.[5] Prevalence of toxoplasmosis varies widely among different regions of the globe and

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depends on meat cooking habits, socioeconomic status, and geographical conditions such as temperature and humidity.[6,7] In Iran, seroprevalence ranged from 14.8% to 66% with typically increasing level according to age, and the overall seroprevalence rate of toxoplasmosis among the Iranian general population was 39.3%.[8]

Prolactin (PRL) hormone is secreted by pituitary gland which is located below the cerebral cortex. Low levels of this hormone are secreted in blood of female and male individuals and the secretion is under control by PRL inhibitory factors such as dopamine.[9] Hyperprolactinemia is a situation in which large amounts of PRL exist in blood of men and pregnant women. The role of PRL has been proven in immune system as PRL receptors are located on the surface of B and T lymphocytes and macrophages and production of cytokines such as tumor necrosis factor alpha (TNF-α), interferon γ (IFNγ), and interleukin-12 (IL-12) are induced by this hormone.[10] The inhibitory effects of PRL on proliferation of *T. gondii* in mononuclear cells of individuals with high levels of PRL have been shown previously.[11] The present study was carried out to assess the possible relation between serum PRL levels and frequency of *T. gondii* infection in humans.

Materials and methods

Study design and patients

Men and women aged 15–58 years with no clinical complications participated in this cross-sectional study. A total of 343 blood samples were collected from individuals who had been referred for PRL measurement in medical diagnostic laboratories in Karaj, Iran, from April to September 2016. Demographic characteristics such as sex, age, marital status, and current pregnancy status were recorded through questionnaires. Woman participants who were pregnant/nursing were excluded from the current study. Then, 3 mL of whole blood samples were collected from each of them; the sera were separated and stored at –20°C until use. After collecting samples, concentration of PRL was measured and the samples were divided into cases with high or low levels of PRL and comparison group with normal levels of PRL.

Serological tests

ELISA was designed to detect anti-*Toxoplasma* IgG antibody in blood sera. The cutoff values of ODs were calculated according to Hillyer et al.[12] The OD of each sample was compared with the cutoff and recorded as positive or negative result. The cutoff value with 95% CI was determined to be 0.45 for the detection of anti-*T. gondii* IgG.

Preparation of soluble antigens of *T. gondii*

Tachyzoites of *T. gondii*, RH strain was maintained in BALB/c mice with serial passages.[13] Tachyzoites that had been inoculated in peritoneal cavity of BALB/c mice were harvested by peritoneal washing with PBS (pH 7.2). The tachyzoites were washed two times with cold PBS, sonicated, and centrifuged at 4°C, 14,000×*g* for 1 hour. Then, supernatant was collected as soluble antigen, and the protein concentration was determined by Bradford method.

Detection of anti-*Toxoplasma* IgG antibody using ELISA technique

Microtiter plates were coated with soluble antigens of *T. gondii*, RH strain. Sera were added in dilution of 1:100 in PBS followed by incubation and washing. Anti-human IgG conjugated with horseradish peroxidase (HRP; Dako Denmark A/S, Glostrup, Denmark) was added after incubation. After washing, chromogenic substrate ortho-phenyline-diamidine (OPD) was added and the reaction was stopped by adding sulfuric acid. The optical density was read and recorded by an automated ELISA reader at 490 nm.[14]

PRL assessment

Concentration of PRL was measured by Roche Elecsys 2010 analyzer, electrochemiluminescence (ECL) technology for all the collected sera according to the manufacturer's instructions. In the first step, 10 µL of the samples were incubated with a biotinylated monoclonal PRL-specific antibody. In the second step, a monoclonal PRL-specific antibody labeled with a ruthenium and streptavidin-coated microparticles were added to the mixture. The reaction mixture was aspirated to a measuring cell and the microparticles were magnetically captured on the surface of an electrode. Unbound substances were removed with ProCell/ProCellM. Chemiluminescence was measured by a photomultiplier and the concentration of PRL was determined via a calibration curve.[15] Interpretation of the PRL concentration was based on the manufacturer's recommendation as follows: normal range for men, 86–324 µIU/mL; and for non-pregnant women, 102–496 µIU/mL. Experiments were carried out in triplicate, and the mean was calculated for each sample.

Ethics approval and consent to participate

The study was carried out according to the principles of Declaration of Helsinki. The current study was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (Approval No: 28451) on December 11, 2015. All animal procedures were carried out according to

the Guidelines for the Care and Use of Laboratory Animals published by the United States National Institutes of Health and approved by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran.

All participants were informed that their participation was voluntary and the method used did not pose any potential risk and their information will be kept strictly confidential. Written informed consent was obtained from all participants before being involved in the study. All participants signed an informed consent and received a complete copy of the signed consent form. In case the person is illiterate, informed consent was obtained by thumbprint and a signature of an impartial witness. Parental consent was obtained from the parents of the minor participants included in this study.

Statistical analyses

Data were analyzed by Statistical Package for Social Sciences software (version 22.0, IBM Corporation, Armonk, NY, USA). Data were analyzed using multiple univariate ANOVA and chi-squared test. Comparison of quantitative variants between two groups was assessed by Student's *t*-test. Data description was carried out by calculating frequencies and 95% CIs. Differences were considered as significant when $P \leq 0.05$.

Results

Distribution of participants

Of the total participants, 70% (240/343) were women and 30% (143/343) men. The highest frequency of participants (152/343, 43%) were found in the age group of 30–39 years.

Seroprevalence of anti-*Toxoplasma* IgG

Of 343 blood serum samples, 110 samples (32%) had anti-*Toxoplasma* IgG. Participants were divided into five age groups of ≤19, 20–29, 30–39, 40–49, and ≥50 years. According to the age of participants, the prevalence of anti-*Toxoplasma* IgG in 343 blood serum samples was as follows: ≤19 age group, 3/16 (18.7%); 20–29, 37/124 (29.8%); 30–39, 53/152 (34.9%); 40–49 age group, 12/38 (31.6%); and ≥50 age group, 5/13 (38.5%) (Tables 1 and 2). Of 240 serum samples of women, 68 (28.3%) had anti-*Toxoplasma* IgG while of 103 serum samples of men 42 (40.8%) had anti-*Toxoplasma* IgG antibody (Tables 1 and 2).

Serum PRL levels

In total, of 343 serum samples, 171 (49.8%) were considered as normal range of PRL, 16 (4.7%) and 156 (45.5%) samples were considered as hypoprolactinemia and hyperprolactinemia, respectively. The detailed data of serum PRL levels according to the sex of participants are shown in Table 3.

Table 1 Frequency of anti-*Toxoplasma* IgG antibody in 240 blood serum samples of women according to particular age groups by ELISA

	***Toxoplasma*-specific IgG**		
	Positive	**Negative**	**Total**
Age groups (years)	n (%)	n (%)	n (%)
≤19	2 (28.6)	5 (71.4)	7 (100)
20–29	27 (27)	73 (73)	100 (100)
30–39	31 (29.2)	75 (70.8)	106 (100)
40–49	5 (23.8)	16 (76.2)	21 (100)
≥50	3 (50)	3 (50)	6 (100)
Total	68 (28.3)	172 (71.7)	240 (100)

Table 2 Frequency of anti-*Toxoplasma* IgG antibody in 103 blood serum samples of men according to particular age groups by ELISA

	***Toxoplasma*-specific IgG**		
	Positive	**Negative**	**Total**
Age groups (years)	n (%)	n (%)	n (%)
≤19	1 (11.1)	8 (88.9)	9 (100)
20–29	10 (41.7)	14 (58.3)	24 (100)
30–39	22 (47.8)	24 (52.2)	46 (100)
40–49	7 (41.2)	10 (58.8)	17 (100)
≥50	2 (28.6)	5 (71.4)	7 (100)
Total	42 (40.8)	61 (59.2)	103 (100)

Table 3 Serum prolactin levels according to sex of the participants by Roche Elecsys 2010 analyzer

Sex	**Prolactin concentration (μIU/mL)**			
	Hypo	**Normal**	**Hyper**	**Total**
	n (%)	n (%)	n (%)	n (%)
Women	12 (5)	96 (40)	132 (55)	240 (100)
Men	4 (3.9)	75 (72.8)	24 (23.3)	103 (100)
Total	16 (4.7)	171 (49.8)	156 (45.5)	343 (100)

Notes: Hypo = hypoprolactinemia (for men: <85; for women: <101) Normal = normal range (for men: 86–324; for women: 102–495), Hyper = hyperprolactinemia (for men: >325; for women: >496).

Association of anti-*T. gondii* IgG antibody and serum PRL levels

According to Tables 4 and 5 the prevalence of *T. gondii* infection in the groups of women and men with high levels of PRL was lower than the comparison group with normal levels of PRL. The statistically significant differences were found between *Toxoplasma* seropositivity in women with high levels of PRL and comparison group (P=0.016), but this difference in men with high levels of PRL was not statistically significant (P=0.74). Furthermore, no statistically significant differences were seen between *Toxoplasma* seropositivity in men and women with low level of PRL and

comparison group (P=1). Moreover, in hyperprolactinemia women, by increasing the PRL levels, the prevalence of *T. gondii* infection decreased.

Discussion

Complex hormonal regulations are necessary for specific immune responses to parasite antigens and effects on interleukins or interferon gamma.[16] Proliferation of lymphocytes in primary and secondary lymphoid organs depends on the interactions between PRL and growth hormone. PRL is a hormone secreted by the pituitary gland which is located below the cerebral cortex.[17] PRL is produced by the placenta uterus, B and T lymphocytes, and NK cells. B and T lymphocytes and macrophages have PRL receptors. PRL secretion is controlled by PRL inhibitory factors, and both men and women have low levels of this hormone in their blood.[18] The situation in which large amounts of PRL are in blood of men or non-pregnant women is called hyperprolactinemia that is fairly common in women.[19] Observed differences between men and women in the prevalence of many parasitic infections can indicate the potential role of sex hormones in the immunity against parasites.[20] One of the hormones that exhibits a wide range of biological activities, including immunomodulatory effects, is PRL. In this study, we have attempted to explain if there was an association between the level of PRL and the frequency of *T. gondii* infections among women and men. Preliminary data, comparing the prevalence of *T. gondii* infection in the population of patients with the PRL level below and above the normal with the population of those having normal PRL level, revealed lower seroprevalence in the group of men and women with hyperprolactinemia. However, differences of *Toxoplasma* seropositivity in women with high levels of PRL was statistically significant in comparison with the population of those having normal levels of PRL (P=0.016). In addition, in hyperprolactinemia women by increasing of PRL levels, the prevalence of *T. gondii* infection decreased. No *Toxoplasma* seropositivity was observed in five serum samples of participants with the highest concentration of PRL.

It has been proven that PRL deficiency in mice may increase the probability and severity of infections. Bromocriptine, the inhibitor of PRL secretion, is used in organ transplantation and autoimmune diseases to inhibit the immune system.[21] It is reported that human PRL has the ability to bind with live tachyzoites of *T. gondii,* RH and ME49 strains.[22] It was shown that PRL has the inhibitory effects on *Toxoplasma* proliferation in mononuclear cells of individuals with high PRL levels. Meli et al in 1996 reported the protective role of PRL against *salmonella typhimurium* in rat model and found that macrophage phagocytic activity and nitric oxide production increased in the rats that had received PRL.[23] Benedetto et al in 2001 showed that PRL can increase the production of

Table 4 Association of anti-*Toxoplasma gondii* IgG antibody and serum prolactin levels in 240 serum samples of women

Prolactin concentration (μIU/mL)	*Toxoplasma*-specific IgG		Total	χ^2 (1 *df*)	*P*-value
	Positive	Negative			
	n (%)	n (%)	n (%)		
Hypo	4 (33.3)	8 (66.7)	12 (100)	0.045	1
Normal	35 (36.5)	61 (63.5)	96 (100)	–	–
Hyper	29 (30)	103 (70)	132 (100)	5.77	**0.016**
Total	68 (28.3)	172 (71.7)	240 (100)		

Notes: Hypo = hypoprolactinemia (for men: <85; for women: <101), Normal = normal range (for men: 86–324; for women: 102–495), Hyper = hyperprolactinemia (for men: >325; for women: >496). Statistical analysis performed by chi-squared analysis. Significance was set at ≤0.05. Positive association is shown in bold.

Table 5 Association of anti-*Toxoplasma gondii* IgG antibody and serum prolactin levels in 103 serum samples of men

Prolactin concentration (μIU/mL)	*Toxoplasma*-specific IgG		Total	χ^2 (1 *df*)	*P*-value
	Positive	Negative			
	n (%)	n (%)	n (%)		
Hypo	2 (50)	2 (50)	4 (100)	0.11	0.74
Normal	31 (41.3)	44 (58.7)	75 (100)	–	–
Hyper	9 (37.5)	15 (62.5)	24 (100)	0.11	1
Total	42 (40.8)	61 (59.2)	103 (100)		

Notes: Hypo = hypoprolactinemia (for men: <85; for women: <101), Normal = normal range (for men: 86–324; for women: 102–495), Hyper = hyperprolactinemia (for men: >325; for women: >496). Statistical analysis performed by chi-squared analysis. Significance was set at ≤0.05.

interleukins 1 and 6 by microglial cells which stimulate anti-*Toxoplasma* function in the brain of infected mice.[24] Zhang et al in 2002 examined two patients with benign pituitary tumors and found *Toxoplasma* cyst among these tumor cells. They reported that multiplication of pituitary cells result in PRL production and anti-*Toxoplasma* activation of microglial cells.[25] Moreover, the hypothesis on the protective role of PRL in protozoan infections is additionally supported by Gomez-Ochoa et al.[26] They concluded that lactating female hamsters that were infected with *Leishmania infantum* showed no symptom of infection compared with control group.[26] Li et al in 2015 showed that PRL-inducible protein (PIP) can impair Th1 immune response and increase susceptibility to *Leishmania major* in mice. PIP is a 14 kDa protein that is present in saliva of mice and upregulates by PRL, and it seems that this protein plays a role in host defense against pathogens.[27] In the study of Serrano et al in 2009 *Neospora* seropositive non-aborting cows had more PRL compared with non-infected ones.[28] Dzitko et al in 2010 suggested the in vitro effects of recombinant PRL on intracellular replication of *T. gondii*, BK strain. It seems that PRL has no direct cytotoxic effects on host cells or parasite, but it can probably bind to parasite surface protein and block its receptors.[29] In the study conducted by Dzitko et al in women with high PRL levels, *T. gondii* prevalence was lower than control group (33.9% vs 45.58%).[30] PRL receptors are located on the surface of B and T lymphocytes and macrophages and the production of cytokines such as TNF-α, IFNγ, and IL-12 is induced by this hormone. The higher levels of TNF-α, IFNγ, and IL-12 in hyperprolactinemia patients may be the reason for protecting these individuals against toxoplasmosis. At the last stage of our analysis, the seroprevalence of toxoplasmosis in women was 28.3% while this value in men reaches to 40.8% (P=0.038), confirming earlier observations carried out on several parasitic diseases. Similar results reported a higher prevalence and intensity of infections for men than for women in the case of protozoan parasites such as *Entamoeba histolytica*, *Leishmania donovani*, *Leishmania braziliensis*, and *Plasmodium falciparum*.[31–34] The overall anti-*Toxoplasma* IgG prevalence was 32% in this study. The prevalence of toxoplasmosis in the general population of this area was 45.5% according to the study by Keshavarz et al in 1998.[35] The seroprevalence of toxoplasmosis among women and men was also estimated in relation to the age of the patients. The highest toxoplasmosis seroprevalence for men and women was found in 30–39 and ≥50 years age group, respectively (Tables 1 and 2). These data were in accordance with the range of general seropositivity expected for Iranian general population.[8]

Conclusion

The results of the current study confirmed the previous studies based on immunoregulatory role of PRL and indicated that high levels of PRL could be related to *T. gondii* seronegativity in women.

Data sharing statement

All data generated or analyzed during this study are not publicly available due to the privacy of the individuals' identities. The dataset supporting the conclusions is available upon request to the corresponding author.

Disclosure

The authors report no conflicts of interest in this work.

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